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Research Article

Evaluation of cypermethrin and *Acorus calamus* L. extract against 4th instar larvae and adults of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.)

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Abstract

The current study was conducted to evaluate bioassay of both *Acorus calamus* Linn. crude hexane extract and Cypermethrin 80SC against the 4th instar larvae and adults of *Aedes albopictus* and *Aedes aegypti* with LC_{50} and LC_{90} values. LC_{50} and LC_{90} values of Cypermethrin on the 4th instar larvae of *A. albopictus* were 0.1470 and 0.6120 ppm respectively while on the *A. aegypti* were 0.0047 and 0.0108 ppm respectively as compared to the *Acorus calamus* extract on the 4th instar larvae of *A. albopictus* were 4.1360 and 22.2766 ppm respectively while on the *A. aegypti* were 5.4420 and 12.4938 ppm respectively. Both Cypermethrin and *Acorus calamus* extract showed a significant difference ($P<0.05$). LC_{50} and LC_{90} values of Cypermethrin on adults of *A. albopictus* were 3.5277 and 5.2844 ppm respectively while on *A. aegypti* were 0.8220 and 3.4267 ppm respectively and a significant difference ($P<0.05$) was to be found as compared to that of *A. calamus* extract on the adults of *A. albopictus* were 52.8842 and 544.1275 ppm respectively while on the *A. aegypti* were 18.5270 and 344.8478 ppm respectively and no significant difference ($P<0.05$) was to be found.

Keywords: *Aedes albopictus; Aedes aegypti; Acorus calamus* extract; Cypermethrin; LC_{50}; LC_{90}

Introduction

The primary vector of dengue in Southeast Asian countries including Pakistan is *Aedes aegypti* (L.) while *Aedes albopictus* (Skuse) is attributed as secondary vector for spreading dengue fever **[1]**. During the last few decades dengue virus infection has become a serious health problem. Annually 55 million people affects due to dengue fever and approximately two fifth of the total population is expected to be at risk of dengue across the world **[2]**. Because of thousands of deaths and every year millions of dengue fever cases, it is known as one of the major public health concern to the urban, suburban and rural tropical areas **[3]**. Fatal diseases are potential threat to children causing by the dengue viruses [4]. About 2.8% deaths occur every year among children in all over the world due to dengue epidemics [5]. *Ae. aegypti* were found in brackish water (BW), an environment which limits the osmotic gradient [6].

A. albopictus and *A. aegypti* are the known suspected vectors of dengue in Lahore, Pakistan [7]. DHF (Dengue Hemorrhage Fever) in Pakistan was first reported in Karachi in 1994 [8]. In 2011, more than 21000 positive cases and above 300 deaths, Lahore have witnessed a severe epidemic of dengue. In Pakistan, the dengue is an alarming situation. Vector control is the only option for having no vaccine and proper antiviral drug to control the disease [9].

It is necessary to know the biology and ecology of vectors to establish a strategic control for mosquitoes [10]. In over populated areas female mosquitoes in a few numbers are enough to cause an outbreak [11]. In all over the world, mosquitoes are known to be insect which affects the health of humans and domestic animals. About 3520 species of mosquitoes which are belonging to 50 different genera are to be found in all over the world, but the most important genera are known to be *Anopheles, Culex and Aedes* which transmit diseases [12]. The intensive migration and urbanization of people results in extended infestation of *Aedes* mosquito, which is known to be principal vector of dengue infections [13].

For the last 2-3 decades, different types of chemical insecticides like organochlorides, organophosphates and carbamates have been used successfully for the control of mosquito vectors. In all over the world insecticide pyrethroid is used for the control of mosquitoes most successfully [14]. The annual use of insecticides from 2000 to 2012, against dengue vectors was 396 tons of organophosphates and 160 tons of pyrethroids [2]. Cypermethrin, a pyrethroid affects the nervous system which causes paralysis in insects [15]. Cypermethrin ultimately causing the death of insects such as mosquitoes when acting on sodium gated channel target site [16].

In future, the most promising mosquito control programs will rely on plant derived chemicals [17]. Thus, the researches for new environmentally safe and target specific insecticide are being conducted. To find new modes of action and also to develop active agents which will be based on natural products, efforts are being made to isolate, screen and develop phytochemicals having pesticidal activity [18].

The traditional approach to initiate dengue control programmes once the dengue virus is introduced into a population and dengue cases received to hospitals makes it difficult to control, evident from the cases all over the world [19].

The economically feasible natural, biodegradable compounds are the inevitable measures for the control of mosquito vector that will replace the expensive synthetic insecticides.

In Multan, from the last few years, the dengue cases are reported from urban, suburban and tropical areas as well. Thus, in current study, focusing Multan, the efficacy of plant extracted *Acorus calamus* Linn (Fam. Araceae) will be evaluated in comparison to the synthetic pyrethroid type-II cypermethrin 80SC against *A. albopictus* and *A. aegypti* in the laboratory.

Materials and methods

Preparation of extraction

Acorus calamus was grinded into smaller size to enhance extraction yield of the rhizome and Soxhlet apparatus was used with hexane for at least 22 hours for extraction. Under vacuum pressure the filtrate was evaporated for dryness. Against the 4th instar larvae and adults of *Ae.*

albopictus and *A. aegypti* the crude hexane extract of *A. calamus* was bioassay.

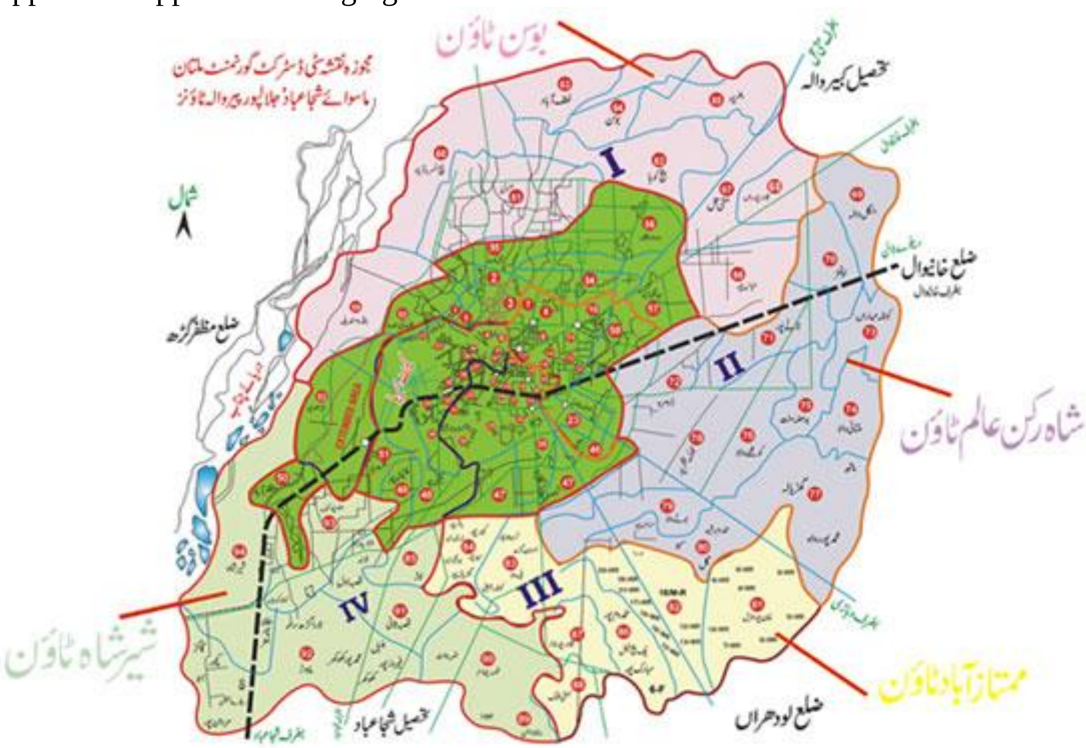
Insecticide

Cypermethrin 80SC was purchased from agricultural market of Multan city.

Bioassay against adults of *Aedes albopictus* and *Aedes aegypyi*

The indoor and outdoor surveillance of larvae of mosquitoes collected from various localities of Shah Rukn-e-Alam Town Multan, (Punjab) Pakistan.

According to WHO [20] standard procedure the bioassay was conducted after some modifications. Laboratory colonies 2-6 days old 20 adult mosquitoes of *A. albopictus* and *A. aegypti* were exposed to filter paper (16X17cm) impregnated with different concentrations of *A. calamus* extract and Cypermethrin 80SC in the range of 0.3220ppm to 6.0ppm for testing against A. *aegypti* and 1.0ppm to 8.0ppm for *A. albopictus* for 2 hours. For Cypermethrin and control impregnated papers each concentration was diluted in 0.1% acetone; by diluting the crude extract with Tween 20 and 0.1% hexane the stock solution for *A. calamus* extract was prepared. For each 1, 3, 5, 10, 20, 30 and 60 minutes, the knockdown was recorded. With the help of clean filter papers all the mosquitoes were then transferred to holding tubes. The mosquitoes were fed in 10% sucrose solution with cotton pads soaked. Each experiment was done in duplicate. After 24 hours, the mortality rate of the adult mosquitoes was recorded. This experiment was repeated three times with the help of Probit Analysis program [21] and SPSS Software the experiment was analyzed.



Bioassay against 4th instar larvae of *A. Albopictus* and *A. aegypti*

According to WHO [22] the bioassay was conducted after some modification. 1000ml glass beakers which contained 250ml of prepared extract of *A. calamus* and Cypermethrin 80SC in different concentrations were prepared to expose 20 *A. albopictus* and *A. aegypti* larvae. In 0.1% hexane the *A. calamus* and control were diluted, while in 0.1% acetone cypermethrin 80SC and control were diluted. The mortality of the larvae was done in duplicate and repeated three times. The percentage mortalities were corrected by Abbott's formula [23] if the control mortality was between 5% and 20%.

$$\frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

By Probit Analysis [22] and SPSS software LC_{50} and LC_{90} values were determined.

Results

The results indicated that the LC_{50} and LC_{90} values of Cypermethrin on 4th instar larvae of *A. albopictus* were 0.1470 and 0.6120 pp while LC_{50} and LC_{90} values of *A. aegypti* larvae of 4th instar were 0.0047 and 0.0180 ppm respectively. A significant difference ($P<0.05$) was to be found on the effect of Cypermethrin to both *A. albopictus* and *A. aegypti*. Similarly, the values of LC_{50} and LC_{90} of *A. calamus* extract on 4th instar larvae of *A. albopictus* were 4.1360 and 22.2766 ppm and the values of LC_{50} and LC_{90} with *A. aegypti* were 5.4420 and 12.4938 ppm respectively. So, a significant difference ($P<0.05$) was also to be found on the effect of *A. calamus* extract on both *A. albopictus* and *A. aegypti* larvae. Cypermethrin showed a significant difference ($P<0.05$) on larvicidal effect to *A. calamus* extract to *A. albopictus* and *A. aegypti* (Table 1).

The results also indicated that the LC_{50} and LC_{90} values of Cypermethrin on *A. albopictus* adults of 3.5277 and 5.2844 ppm while LC_{50} and LC_{90} values with *A. aegypti* 0.8220 and 3.4267 ppm respectively. A significant difference ($P<0.05$) was to be found on the effect of Cypermethrin to *A. albopictus* and *A. aegypti* adults. Similarly, the values of LC_{50} and LC_{90} of *A. calamus* extract were 52.8842 and 54.1275 ppm on *A. albopictus* while the values of LC_{50} and LC_{90} on *A. aegypti* were 18.5270 and 344.8478 ppm respectively. However, there was no significant difference ($P>0.05$) on the effect of *A. albopictus* and *A. aegypti* to be effective as on adulticide as compared to that of Cypermethrin (Table 2).

Table 1. The LC_{50} and LC_{90} of Cypermethrin and *Acorus calamus* extract on *Aedes albopictus* and *Aedes aegypti* 4th instar larvae in the laboratory

Treatment	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± SE	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± SE
	Aedes albopictus			*Aedes aegypti*		
Cypermethrin	0.1470	0.6120	3.3381±0.3844	0.0047	0.0108	3.7938±0.2774
Acorus calamus extract	4.1360	22.2766	6.6702±0.7832	5.4420	12.4938	4.1441±0.4682

Table 2. The LC_{50} and LC_{90} of Cypermethrin and *Acorus calamus* extract on *Aedes albopictus* and *Aedes aegypti* adults in the laboratory

Treatment	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± SE	LC_{50} (ppm)	LC_{90} (ppm)	Slope ± SE
	Aedes albopictus			*Aedes aegypti*		
Cypermethrin	3.5277	5.2844	6.2887±0.8220	0.8220	3.4267	3.4812±0.3884
Acorus calamus extract	52.8842	544.1275	1.2982±0.1441	18.5270	344.8478	1.2047±0.1314

Discussion

For human health dengue virus has now become a dangerous problem and no proper vaccine is available for treatment and the only option for the control of vector is to control dengue infection [24]. For larvicidal activities against mosquitoes methanol extracts of some plants were used in Malaysia. To all mosquitoes species of *Culex quinquefasciatus* like, *A. albopictus*

(Skuse), *A. aegypti* (L.) and *Anopheles maculatus* Theobald with LC_{50} of 38.16 – 59.30 ug/ml, the methanol extract of *A. calamus* Linn. showed a high degree of toxicity **[25]**. By using the hexane fraction, the highest larvicidal effect on *A. aegypti* 4th instar larvae with LC_{50} value of 1.89 ppm and the LC_{90} value of 10.86 ppm respectively was found **[25]**. The present study using the hexane fraction indicated LC_{50} of 4.1360 ppm and LC_{90} value of 22.2766 respectively against 4th instar larvae of *A. albopictus*, while the LC_{50} and LC_{90} values of *A. aegypti* 4th instar larvae were to be found 5.4420 ppm and 12.4938 ppm respectively. Some studies have found the volatile oil of curcuma aromatic (Fam: Zingiberaceae) more significant larvicidal activity against 4th instar larvae of *A. aegypti* than the extracts of hexane fraction with LC_{50} and LC_{90} values 37.29 ppm and 58.16 ppm respectively **[26]**. In another study, the seed extract of *Apium graveolans* (Fam: Apiaceae) also showed larvicidal activity against 4th instar larvae of *A. aegypti* with LD_{50} and LD_{95} values of 82.1 and 177.9 mg/L (ppm), respectively **[25]**. Essential oil in the laboratory bioassay of *Ipomoea cairica* (Fam: Convoloceae) against *A. aegypti* larvae LC_{50} and LC_{90} values were found to be 23.3 and 93.8ppm respectively **[27]**. The present study indicated that the extract of *A. calamus* is more effective as compared to than that of the extracts of *Ipomoea cairiaca*, *Curuma aromatic* and *Apium graveolans* as larvicidal against 4th instar larvae of *A. aegypti* evaluated by the above authors mentioned.

From methanol extract of *A. calamus* rhizome by using the hexane fraction against *A. aegypti* adults in some study was found to be the most effective and exhibiting LC_{50} value of 0.05mg/cm^2 and LC_{90} values of 0.08 mg/cm^2. Good adulticidal property also displayed for *Litsea elliptica* the methanol fraction with LC_{50} value of 0.12 mg/cm^2 and LC_{90} value of 7.09 mg/cm^2 **[28]**.

A. calamus extract and bifenthrin were evaluated at high rise flats in Kuala Lumpur The weekly impact of both insecticides and plant extract on field populations of *Ae. albopictus* and *A. aegypti* was recorded. *A. calamus* extract caused 94.8% (inside flats) to 95.7% (outside flats) adult *A. aegypti* mortalities as compared to that of bifenthrin adult mortalities with 97.4% (inside flats) and 99.2% (outside flats). In the control group the mortalities of the adults of *A. aegypti* were 20.3% (inside flats) and 19.4% (outside flats) respectively 24 hour after spraying of ULV **[29]**. In some studies the adulticidal efficacy of the crude seed extract of *Apium graveolans* evaluated against *A. aegypti* and found to be a slightly adulticidal potency of this type of extract with LD_{50} value of 6.8 and LD_{95} value of 68.8 mg / cm^2 **[30]**. Adulticidal activity of hexane –extracted *Curcuma aromatic* was also be tested in another studies and it was found to be slightly more effective with the value of LC_{50} of 1.62 ug/mg as compared to volatile oil with the value of LC_{50} of 2.96 ug/mg respectively **[26]**. In another studies in testing the adulticidal activity, *A. calamus* extract exhibited the LC_{50} value of 17.4176 ppm and LC_{90} value of 253.9558 ppm against *A. aegypti* and a higher value of LC_{50} of 44.9852 ppm and LC_{90} value of 447.1466 ppm on *A. albopictus*. In the 4th instar larvicidal crude hexane extract showed value of LC_{50} of 0.4518 ppm and value of LC_{90} of 12.3835 ppm **[31]**.

Conclusion

The current study indicated that the Cypermethrin has more toxic effect on *Aedes* species larvae as well as on adults as compared to that of the *Acorus calamus* extract, but this plant extract could be utilized for dengue control in search for botanical insecticide. The wide availability of this plant in tropical areas can be exploited for vector control in such type of usage.

Authors' contributions

Conceived and designed the experiments: M Zafar, Performed the Experiments: M Zafar, Analyzed the Data: MKA Khan, I Mushtaq & Asmatullah, Contributed reagents/ materials/ analysis tools: M Zafar, MKA Khan, I Mushtaq & Asmatulah, Wrote the paper: M Zafar.

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